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Escherichia coli bacteriuria in female adults is associated with the development of hypertension

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ABSTRACT

Objective: To investigate whether *Escherichia coli* bacteriuria is associated with the development of hypertension during a long-term follow-up.
Methods: A prospective cohort study was performed among the participants of two population-based studies. Between 1974 and 1986 all women aged 39 to 68 years old, who lived in Utrecht, the Netherlands, were invited to participate in a breast cancer screening program. The participants completed a questionnaire, underwent a medical examination, and collected a morning urine sample that remained stored. From 1993 to 1997 another population-based study was performed. We performed a full cohort analysis for 444 women who participated in both studies. *E. coli* bacteriuria was diagnosed by a real-time PCR. Hypertension was defined as the use of antihypertensive medication and/or a measured systolic blood pressure of at least 160 mmHg or a diastolic blood pressure of 95 mmHg or higher. The mean follow-up was 11.5 ± 1.7 years.
Results: Forty women (9%) had *E. coli* bacteriuria at baseline. Women who had bacteriuria at baseline had a mean blood pressure at study endpoint of 133 ± 20 mmHg systolic and 78 ± 11 mmHg diastolic, and women without bacteriuria had values of 129 ± 20 and 78 ± 11 mmHg, respectively (*p*-values for difference 0.33 and 0.88). Although *E. coli* bacteriuria was not associated with the blood pressure as a continuous variable, it was associated with the development of hypertension during follow-up (OR 2.8, 95% CI 1.4–5.5).
Conclusion: E. coli bacteriuria may increase the risk of future hypertension.

1. Introduction

Although the urinary tract is normally sterile, asymptomatic bacteriuria is a common phenomenon, especially in women. Different studies report a prevalence of asymptomatic bacteriuria of approximately 5% among healthy young women, increasing to over 20% in the elderly.[1,2] *Escherichia coli* is the most prevalent uropathogen.[3] Several authors in the first half of the twentieth century have suggested a role of bacteriuria in the etiology of hypertension.[4] Although more recent studies have also found a correlation, no prospective study has convincingly shown that bacteriuria itself leads to hypertension.[5]

The present study aimed to address the question of whether or not *E. coli* bacteriuria is associated with an increased risk of the development of hypertension during a long-term follow-up period. In addition, the risk of a heart attack or stroke in the presence of bacteriuria was studied.

2. Methods

2.1. Study population

A full cohort analysis was performed for women who participated in two population-based studies. Between 1974 and 1986, all women born between 1911 and 1945, who lived in the city of Utrecht and surrounding area, were invited for a breast cancer screening program, with a participation rate of 68–72%.[6] A total number of 38 994 women aged 39 to 68 years at intake participated (the baseline cohort). Baseline measurements, performed between 1974 and 1986, included extensive questionnaires, a short medical examination, and the collection of a midstream morning urine sample. Data obtained through the questionnaires included age, marital status, smoking habits, parity, menopausal age, diet, and drug use. During the medical examination weight and height were measured. Approximately 200 ml

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urine was stored in plastic polypropylene jars, without preserving agents, and stored at −20 °C for future analyses.[7] All women gave oral consent to use their data and urine samples for future scientific research.

From 1993 to 1997, participants in the breast cancer screening program received an invitation by mail to join an additional study to assess the relationship between nutrition and cancer and other chronic diseases, the Prospect-EPIC study (the follow-up cohort). A total of 17 357 women living in Utrecht and the surrounding area agreed to take part (participation rate 34.5%).[8] Participants were between 49 and 70 years of age at enrolment. Information was collected on the basis of two self-administered questionnaires and a medical examination including blood pressure. Non-fasting blood samples were successfully drawn from 97.5% of the women and stored under liquid nitrogen at −196 °C. The serum creatinine level was later measured from these samples. Approximately 88% of the women signed a detailed informed consent, enabling the researchers to use their blood samples for future analysis, and to obtain information on future morbidity and mortality.[8]

In total, 506 women participated in both the baseline cohort and the follow-up cohort. Sixty-two women had to be excluded for the following reasons: a missing urine sample ($n = 13$), the use of antihypertensive medication at baseline ($n = 45$), kidney transplantation during follow-up ($n = 1$), or missing data on hypertension at study endpoint ($n = 3$). Finally, 444 women were included in the prospective study to assess the relationship between bacteriuria and the development of hypertension. The mean duration of follow-up was 11.5 ± 1.7 years, ranging from 8.3 to 18.6 years from baseline until participation in the follow-up study (and was not different for women with compared to women without bacteriuria).

This study was approved by the Medical Ethics Committee of the University Medical Center Utrecht, the Netherlands.

2.2. Escherichia coli bacteriuria

E. coli bacteriuria was defined as the presence of 10^5 colony forming units (cfu) of *E. coli* per ml of urine. It was diagnosed by a real-time PCR that we developed and validated beforehand, a technique that has also been used by others.[9,10] Briefly, PCR primers and probe complementary to regions of the *gadA* gene specific for *E. coli* were designed for the real-time PCR assay. The laboratory sensitivity and specificity of the real-time PCR tested with 50 *E. coli* strains and with 41 non-*E. coli* strains (including the most prevalent uropathogens and many members of the vaginal and anal flora) were 100% (50/50) and 98% (40/41), respectively. For clinical evaluation, 42 clinical urine specimens (12 with and 30 without *E. coli*) were tested and the results were compared to those of a clinical conventional urine culture. The sensitivity and specificity of the real-time PCR in these clinical samples were 92% and 87%, respectively. The test results were quantitative and allowed distinguishing between significant bacteriuria (i.e., 10^5 cfu/ml) and low-count bacteriuria that might have been due to contamination.

To test a study urine sample, 1 ml of urine was centrifuged at 16 250 × *g* for 5 min. The pellet was washed twice, suspended in 1 ml of sterile injection water, and 100 μl of the suspension was heated for 2 min at 1000 Watt in a microwave oven for DNA preparation. Five microliters was added to the real-time PCR reaction volume as DNA template. Each 25 μl reaction volume consisted of 12.5 μl 2 × TaqMan Universal PCR Master Mix (Applied Biosystems, Branchburg, New Jersey, USA) that contains AmpliTaq Gold DNA polymerase, 300 nM forward primer (5′-ACCGACATCGTGGTGATGC-3′), 300 nM reverse primer (5′-AGCAA-CAGTTCAGCAAAGTCCA-3′), and 175 nM probe (5′-CAT-TATGTGTCGTCGCGGCTTCGAA-3′); the DNA template was the last ingredient added. The ABI PRISM 7700 Sequence Detection System (PE Biosystems, Nieuwerkerk aan de IJssel, the Netherlands) was used for the real-time PCRs. Cycling parameters were first the uracil-*N*-glycosylase (UNG) reaction at 50 °C for 2 min, then AmpliTaq Gold activation at 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 15 s – combined annealing and extension at 60 °C for 1 min. Emitted fluorescence from each well was measured during both the denaturation and annealing/extension steps in every cycle. Amplification plots were constructed using the ABI PRISM 7700 Sequence Detection System software, version 1.7 (PE Biosystems).

2.3. Blood pressure, heart attack, stroke

Blood pressure was measured with a standard mercury sphygmomanometer after the subject had been seated for 5 min. Hypertension was defined as the previous use of antihypertensive medication (assessed at follow-up by the question: "Have you ever been treated with drugs for high blood pressure?") and/or a measured systolic blood pressure of at least 160 mmHg or a diastolic blood pressure of 95 mmHg or higher.[11] A history of having had a heart attack or stroke was assessed at follow-up by the two additional questions: "Have you ever had a heart attack?", "Have you ever had a stroke?".

2.4. Data analysis

The present results are based on a cohort study of 444 women who were followed for the development of hypertension in relation to *E. coli* bacteriuria at baseline.

Baseline characteristics were compared between women with and without bacteriuria. Comparisons between means were performed with the Student's *t*-test and comparisons between nominal or categorical data with the Chi-square test. Linear regression analysis was used to calculate the adjusted difference in blood pressure between women with versus women without bacteriuria. The relative risk of hypertension in the presence of bacteriuria was estimated by logistic regression and quantified as odds ratios (OR) and 95% confidence intervals (CI). Adjustment was done for potential confounding factors, i.e., age, weight, and serum creatinine level.

Table 1
Baseline characteristics for the total cohort study, and separately for women with versus without *E. coli* bacteriuria at baseline.

	Total study group ($N = 444$)[a]	No *E. coli* bacteriuria ($n = 404$)	*E. coli* bacteriuria ($n = 40$)	*p*-Value
Age, years	44.9 ± 3.2	44.9 ± 3.2	45.3 ± 3.4	0.51
Body mass index, kg/m^2	24.0 ± 3.2	23.9 ± 3.2	24.6 ± 3.6	0.26
Postmenopausal	98 (22%)	88 (22%)	10 (25%)	0.64
Married or living with partner ($N = 336$)	303 (90%)	273 (90%)	30 (97%)	0.20
Given birth to living child(ren)	401 (90%)	363 (90%)	38 (95%)	0.29
Antibiotics ($N = 108$)	13 (12%)	10 (10%)	3 (33%)	0.04

Values are given as mean ± standard deviation or as number of patients (percentage).
[a] The total number of study subjects is 447 unless otherwise stated.

Table 2
Follow-up characteristics of the cohort study divided into women with and without *E. coli* bacteriuria ($N = 444$ unless otherwise stated).

	No *E. coli* bacteriuria ($n = 404$)	*E. coli* bacteriuria ($n = 40$)	OR (95% CI)	*p*-Value	Adjusted *p*-value[a]
Hypertension[b]	78 (19%)	16 (40%)	2.8 (1.4–5.5)	0.003	0.003
Antihypertensive medication	49 (12%)	10 (25%)	2.4 (1.1–5.2)	0.03	0.03
Systolic BP ≥160 and/or diastolic BP ≥95 mmHg	45 (11%)	9 (23%)	2.3 (1.0–5.2)	0.04	0.04
Systolic BP > 140 and/or diastolic BP > 90 mmHg	112 (28%)	14 (35%)	1.4 (0.7–2.8)	0.33	0.40
Heart attack ever ($N = 443$)	4 (1%)	1 (3%)	2.6 (0.3–23.5)	0.41	0.59
Stroke ever	7 (2%)	1 (3%)	1.5 (0.2–12.1)	0.73	0.72

OR, odds ratio; CI, confidence interval; BP, blood pressure.
Values are given as mean ± standard deviation or as number of patients (percentage).
[a] Adjustments were made for age, weight, and serum creatinine.
[b] For definition see Methods section.

3. Results

Baseline characteristics of the total study group (444 women) are presented in Table 1, as well as the comparisons between women with ($n = 40$, 9%) and without ($n = 404$, 91%) *E. coli* bacteriuria at baseline. The use of antibiotics was the only factor that significantly differed between women with and without bacteriuria (33% vs. 10%, $p = 0.04$). Eight of the 45 women (18%) who had to be excluded because of the use of antihypertensive medication at baseline had *E. coli* bacteriuria, which was higher than the percentage of 9% of the final study group without antihypertensive drugs at baseline ($p = 0.06$).

At measurement after a mean follow-up of 11.5 years, women who had *E. coli* bacteriuria at baseline had a mean blood pressure at study endpoint of 133 ± 20 mmHg systolic and 78 ± 11 mmHg diastolic, and women without bacteriuria had values of 129 ± 20 and 78 ± 11 mmHg, respectively (*p*-value for difference 0.33 and 0.88). Although *E. coli* bacteriuria was not associated with the blood pressure as a continuous variable, it was associated with the development of hypertension (OR 2.8, 95% CI 1.4–5.5) (Table 2). This was mainly due to more bacteriuric women who started antihypertensive drugs when compared to non-bacteriuric participants. The association remained statistically significant after correction for age, weight, and creatinine (OR 2.8, 95% CI 1.4–5.6).

In a subgroup analysis of the 108 women for whom it was known whether or not they had used antibiotics at baseline, the same trend was visible, also after correction for antibiotic use, although it lost statistical significance (data not shown). *E. coli* bacteriuria was not associated with the different classes of antihypertensive drugs (data not shown). The incidence of heart attacks and strokes was not increased among women with bacteriuria at baseline (Table 2).

4. Discussion

In this prospective cohort study, in a population of healthy adult women, we found a correlation between *E. coli* bacteriuria and the prevalence of hypertension 12 years later.

Among the strengths of our study are its size and the length of follow-up. A final cohort of 444 women for whom a urine sample was stored at baseline could be followed for as long as 12 years, which gave us the unique opportunity to study bacteriuria and its long-term consequences.

The limitations include the fact that we had to rely on only one urine sample to define bacteriuria. However we, and others, have validated this before.[12] We made the assumption that however bacteriuria might be transient in a proportion of the bacteriuric study subjects, bacteriuria at one point reflects a higher susceptibility to recurrent and persistent bacteriuria in general, even after antimicrobial therapy. Previous findings are supportive of this assumption.[1,13] Some of the urine samples may have become contaminated before storage, however this will be equally divided in the total study group. Moreover, contamination usually leads to the growth of more pathogens, often non-*E. coli*, with lower colony counts, which are not picked up by our real-time PCR. *E. coli* is the causative microorganism found to be most prevalent, and the prevalence of *E. coli* bacteriuria of 9% among middle-aged women reported here is in the range of what could be expected beforehand. There are no published data that can be cited to support the test characteristics of real-time PCR on urine stored for more than 10 years. However, it seems plausible that the *gadA* gene for *E. coli* should still be present also after a long time period. We did test urine samples that were stored for up to 5 years and compared the results to those of the conventional urine culture that was performed at the time the samples were fresh (unpublished data). The results were similar to those from the fresh samples. The baseline data included the use of any drugs, which allowed us to exclude women who used antihypertensive medication. However, blood pressure was not measured at baseline, and therefore the study cohort will include some women with undiagnosed hypertension. At follow-up we had to rely on a single blood pressure measurement. But we assume that the incidence of increased blood pressure due to other reasons will be equally divided among women with and without bacteriuria at baseline. Moreover, the increased prevalence of hypertension in the group of bacteriuria was mainly due to more women who started antihypertensive drugs in this group compared to the group of women without bacteriuria. Unfortunately, data on the prevalence of diabetes mellitus at baseline were lacking, and therefore adjustment for diabetes was not performed.

Several authors in the first half of the twentieth century have suggested a role of bacteriuria in the etiology of hypertension, as reviewed before.[4] For instance, Kass showed small differences in blood pressure between bacteriuric and non-bacteriuric women aged 15 to 64 years.[14] Although more recent studies have also found a correlation, no prospective study has convincingly shown that bacteriuria itself leads to hypertension.[5] In our cohort study, we found a higher prevalence of hypertension in the bacteriuric group after 12 years of follow-up. The underlying mechanism of this finding is not clear. Hypertension is a lasting increase in blood pressure with a heterogeneous etiology consisting of both genetic and environmental factors. Patients share the inability to excrete sodium at a normal arterial pressure.[15] If bacteriuria leads to hypertension, the most attractive explanation would be that hypertension arises secondary to renal scarring caused by the type 1 fimbriae of the uropathogens. In the multivariate analysis, correction for creatinine did not change the results, but hypertension can occur before the reduction in creatinine clearance becomes apparent (for example in chronic glomerulonephritis).[16] The absence of a change in odds ratio after adjustment for age, weight, and creatinine confirms the notion that these factors were not related to bacteriuria. An alternative explanation is that both bacteriuria and hypertension are found more frequently among individuals with co-morbidity or that they share a same (currently

unknown) cause. This is supported by the higher prevalence of bacteriuria among women who used antihypertensive drugs at baseline. It remains unclear why *E. coli* bacteriuria was associated with the development of hypertension but not with the blood pressure as a continuous variable. However, it seems plausible that part of it is due to the success of antihypertensive drugs.

The percentage of women who developed hypertension during follow-up was in agreement with what could be expected. A large population-based study performed in the Netherlands also between 1993 and 1997, showed a prevalence of hypertension in women in the same age group of 15%.[17]

In conclusion, in this prospective study a strong correlation was found between *E. coli* bacteriuria and hypertension after 12 years of follow-up. Given the importance of hypertension the nature of this correlation needs to be studied further.

Conflict of interest: No conflict of interest to declare.

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